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(54) Title: RADIOLABELED COMPOUNDS FOR THROMBUS IMAGING (57) Abstract This invention relates to radiolabeled scintigraphic imaging agents, and methods and reagents for producing such agents. Specifically, the invention relates to specific binding compounds, including peptides, that bind to a platelet receptor that is the platelet GPIIb/IIIa receptor, methods and kits for making such compounds, and methods for using such compounds labeled with technetium-99m via a covalently-linked radiolabel-binding moiety to image thrombi in a mammalian body.		

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RADIOLABELED COMPOUNDS FOR THROMBUS IMAGING**BACKGROUND OF THE INVENTION****1. Field of the Invention**

5 This invention relates to scintigraphic imaging agents and reagents, and methods for producing such agents and reagents. Specifically, the invention relates to reagents that can be radiolabeled with technetium-99m (Tc-99m), methods and kits for making and radiolabeling such reagents, and methods for using such radiolabeled reagents to image sites of thrombus formation in a mammalian body.

2. Description of the Prior Art

10 Thrombosis and thromboembolism, in particular deep vein thrombosis (DVT) and pulmonary embolism (PE), are common clinical conditions that are associated with significant morbidity and mortality. It has been estimated that in the U.S.
15 approximately 5 million patients experience one or more episodes of DVT per year and that over 500,000 cases of pulmonary embolism occur, resulting in 100,000 deaths (J. Scabold, Society of Nuclear Medicine Annual Meeting 1990). It has also been estimated that over 90% of all pulmonary emboli arise from DVT in the lower extremities. Anticoagulant therapy can effectively treat these conditions if applied
20 early enough. However, such treatment is associated with risks (e.g. internal bleeding) that prevent unnecessary prophylactic application. More advanced techniques of thrombolytic intervention (such as the administration of recombinant tissue plasminogen activator or streptokinase) can be used in acute cases, but these techniques carry even greater risk. Moreover, effective clinical application of these
25 techniques requires that the site of the offending thrombus be identified so as to monitor the effect of treatment.

30 For these reasons, a rapid means of localizing thrombi *in vivo*, most preferably using non-invasive methods, is highly desirable. Methods currently utilized for the identification of sites of deep-vein thrombosis are contrast venography and compression B-mode ultrasound; the choice of which technique is used depends on the expected location of the thrombus. However, the former technique is invasive and both techniques are uncomfortable for the patient. In addition, these methods are in many cases either unsuitable or yield inaccurate results.

Current methods used to diagnose PE include chest X-ray, electrocardiogram (EKG), arterial oxygen tension, perfusion and ventilation lung scans, and pulmonary angiography. Apart from the latter (invasive) procedure, none of these methods is capable of providing an unequivocal diagnosis.

5 In the field of nuclear medicine, certain pathological conditions are localized, or their extent is assessed, by detecting the distribution of small quantities of internally-administered radioactively labeled tracer compounds (called radiotracers or radiopharmaceuticals). Methods for detecting these radiopharmaceuticals are known generally as imaging or radioimaging methods.

10 A variety of radionuclides are known to be useful for radioimaging, including ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$ (Tc-99m), ^{111}In , ^{123}I , ^{125}I , and ^{169}Yb . Of these radionuclides, Tc-99m and ^{111}In are preferred single photon-emitting radionuclides and ^{68}Ga is preferred as a positron-emitting radionuclide. Tc-99m is a preferred radionuclide because it does not emit alpha or beta particle radiation and emits gamma radiation at about 140
15 keV, has a physical half-life of 6 hours, and is readily available on-site using a molybdenum-99/technetium-99m generator.

A gamma-emitting radiotracer that binds specifically to a component of a thrombus in preference to other tissues when administered *in vivo* can provide an external scintigraphic image which defines the location of the thrombus-bound
20 radiotracer and hence the thrombus. Thrombi are constructs of blood cells (largely activated platelets) enmeshed in cross-linked fibrin. Activated platelets are particularly good targets for radioimaging thrombi because they are not normally found in circulating blood (which contains unactivated platelets).

Activated platelets express the GPIIb/IIIa receptor on their cell surfaces. The
25 normal ligand for this receptor is fibrinogen (Plow *et al.*, 1987, Perspectives in Inflammation, Neoplasia and Vascular Cell Biology, pp. 267-275). However, small, synthetic analogues, which may be but are not necessarily peptides, have been developed that bind to this receptor (examples include Klein *et al.*, 1992, U.S. Patent No. 5,086,069 and Egbertson *et al.*, 1992, European Patent Application No. EPA
30 0478328A1). Although many of these synthetic molecules bind with only low affinity, others have been made that have very high affinity (*see* Egbertson *et al.*, *ibid.*).

Attempts to provide radiotracers for imaging thrombi are known in the prior art. These include autologous platelets, labeled with either ^{111}In or $^{99\text{m}}\text{Tc}$ (Tc-99m), and ^{123}I - and ^{125}I -labeled fibrinogen (the latter detected with a gamma scintillation probe as opposed to a gamma camera). Additional radiolabeled compounds used to label thrombi include plasmin, plasminogen activators, heparin, fibronectin, fibrin Fragment E₁ and anti-fibrin and anti-platelet monoclonal antibodies (*see* Knight, 1990, *Sem. Nucl. Med.* 20: 52-67 for review).

Compounds having the ability to bind to the platelet GPIIb/IIIa receptor are known in the prior art.

10 Ruoslahti & Pierschbacher, U.S. Patent No. 4,578,079 describe peptides of sequence X-Arg-Gly-Asp-R-Y, wherein X and Y are either H or an amino acid, and R is Thr or Cys, the peptides being capable of binding to platelets.

Ruoslahti & Pierschbacher, U.S. Patent No. 4,792,525 describe peptides of sequence Arg-Gly-Asp-X, wherein X is Ser, Thr or Cys, the peptides being capable of binding to platelets.

15 Klein *et al.*, 1992, U.S. Patent No. 5,086,069 disclose guanine derivatives that bind to the GPIIb/IIIa receptor.

Pierschbacher *et al.*, 1989, PCT/US88/04403 disclose conformationally-restricted RGD-containing peptides for inhibiting cell attachment to a substratum.

20 Nutt *et al.*, 1990, European Patent Application 90202015.5 disclose cyclic RGD peptides that are fibrinogen receptor antagonists.

Nutt *et al.*, 1990, European Patent Application 90202030.4 disclose cyclic RGD peptides that are fibrinogen receptor antagonists.

25 Nutt *et al.*, 1990, European Patent Application 90202031.2 disclose cyclic RGD peptides that are fibrinogen receptor antagonists.

Nutt *et al.*, 1990, European Patent Application 90202032.0 disclose cyclic RGD peptides that are fibrinogen receptor antagonists.

Nutt *et al.*, 1990, European Patent Application 90311148.2 disclose cyclic peptides that are fibrinogen receptor antagonists.

30 Nutt *et al.*, 1990, European Patent Application 90311151.6 disclose cyclic peptides that are fibrinogen receptor antagonists.

Ali *et al.*, 1990, European Patent Application 90311537.6 disclose cyclic

peptides that are fibrinogen receptor antagonists.

Barker *et al.*, 1991, PCT/US90/03788 disclose cyclic peptides for inhibiting platelet aggregation.

5 Pierschbacher *et al.*, 1991, PCT/US91/02356 disclose cyclic peptides that are fibrinogen receptor antagonists.

Duggan *et al.*, 1992, European Patent Application 92304111.5 disclose fibrinogen receptor antagonists.

Garland *et al.*, 1992 European Patent Applications 92103861.8 and 92108214.5 disclose phenylamide derivatives as platelet aggregation inhibitors.

10 Bondinell *et al.*, 1993, International Patent Application Serial No. PCT/US92/05463 disclose bicyclic fibrinogen antagonists.

Blackburn *et al.*, International Patent Application Serial No. PCT/US92/08788, disclose nonpeptidyl integrin inhibitors having specificity for the GPIIb/IIIa receptor.

15 Egbertson *et al.*, 1992, European Patent Application 0478328A1 disclose tyrosine derivatives that bind with high affinity to the GPIIb/IIIa receptor.

Ojima *et al.*, 1992, 204th Meeting, Amer. Chem. Soc. Abst. 44 disclose synthetic multimeric RDGF peptides useful in inhibiting platelet aggregation.

20 Hartman *et al.*, 1992, *J. Med. Chem.* 35: 4640-4642 describe tyrosine derivatives that have a high affinity for the GPIIb/IIIa receptor.

Radiolabeled peptides for radioimaging thrombi have been reported in the prior art.

25 Stuttle, 1990, PCT/GB90/00933 discloses radioactively labeled peptides containing from 3 to 10 amino acids comprising the sequence arginine-glycine-aspartic acid (RGD), capable of binding to an RGD binding site *in vivo*.

Rodwell *et al.*, 1991, PCT/US91/03116 disclose conjugates of "molecular recognition units" with "effector domains".

30 The use of chelating agents for radiolabeling peptides, and methods for labeling peptides with Tc-99m are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/653,012, 07/807,062, 07/871,282, 07/886,752, 07/893,981, 07/955,466, 08/019,864, 08/073,577, 08/210,822, 08/236,402 and 08/241,625, and radiolabeled peptides for use as scintigraphic

imaging agents for imaging thrombi are known in the prior art and are disclosed in co-pending U.S. Patent Applications Serial Nos. 07/886,752, 07/893,981 and 08/044,825 and International Patent Applications Serial Nos. PCT/US92/00757, PCT/US92/10716, PCT/US93/02320, PCT/US93/03687, PCT/US93/04794, PCT/US93/05372, PCT/US93/06029, PCT/US93/09387, PCT/US94/01894, PCT/US94/03878, and PCT/US94/05895, each of which are hereby incorporated by reference in its entirety.

There remains a need for small (to enhance blood and background tissue clearance), synthetic (to make routine manufacture practicable and to ease regulatory acceptance), high-affinity, specific-binding molecules radiolabeled with a convenient radiolabel, preferably Tc-99m, for use in imaging thrombi *in vivo*. Small synthetic compounds that bind specifically to the GPIIb/IIIa receptor on activated platelets, that are radiolabeled with a convenient radioisotope, preferably Tc-99m, ¹¹¹In or ⁶⁸Ga, fulfill this need in the art, and are provided by this invention.

SUMMARY OF THE INVENTION

This invention provides small, synthetic, radiolabeled (preferably Tc-99m, ¹¹¹In or ⁶⁸Ga labeled) compounds that bind to the GPIIb/IIIa receptor with high affinity, as scintigraphic agents for non-invasive imaging of thrombi *in vivo*. The invention thereby provides scintigraphic thrombus imaging agents that are radioactively-labeled reagents. Specifically, the invention provides reagents for preparing thrombus imaging agents that are radiolabeled with technetium-99m (Tc-99m), ¹¹¹In or ⁶⁸Ga, preferably with Tc-99m. The reagents of the invention are each comprised of a specific binding compound, including but not limited to peptides, that binds specifically and with high affinity to the platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor, and is covalently linked to a radiolabel-complexing moiety.

We have found that, for optimal imaging, the reagent must be capable of binding to the platelet GPIIb/IIIa receptor with sufficient affinity that it inhibits the adenosine diphosphate (ADP)-induced aggregation of human platelets in a standard platelet aggregation assay (*see Example 3 below*) to the extent of 50% when present at a concentration of no more than 1 μ M.

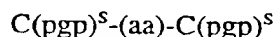
It is of distinct commercial advantage to use small compounds, preferably

having a molecular weight of less than about 10,000 daltons. Such small compounds can be readily manufactured. Moreover, they are likely not to be immunogenic and to clear rapidly from the vasculature, thus allowing for better and more rapid imaging of thrombi. In contrast, larger molecules such as antibodies or fragments thereof, or other biologically-derived peptides larger than 10,000 daltons, are costly to manufacture, and are likely to be immunogenic and clear more slowly from the bloodstream, thereby interfering with rapid diagnoses of thrombi *in vivo*.

The invention also provides reagents wherein the specific binding compounds are linear or cyclic peptides having an amino acid sequence of 4 to 100 amino acids and a molecular weight no greater than about 10,000 daltons.

One aspect of the invention provides a reagent for preparing a thrombus imaging agent that is capable of being radiolabeled for imaging thrombi within a mammalian body, comprising a specific binding compound that specifically binds to the platelet GPIIb/IIIa receptor, and that is covalently linked to a Tc-99m complexing moiety of formula:

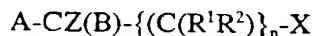
I.



wherein $C(\text{pgp})^S$ is a protected cysteine and (aa) is any primary α - or β -amino acid not containing a thiol group. In a preferred embodiment, the amino acid is glycine.

In another embodiment, the invention provides a reagent for preparing a thrombus imaging agent that is capable of being radiolabeled for imaging thrombi within a mammalian body, comprising a specific binding compound that specifically binds to the platelet GPIIb/IIIa receptor, that is covalently linked to a Tc-99m complexing moiety comprising a single thiol-containing moiety of formula:

II.



wherein A is H, HOOC, H_2NOC , (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4 ; B is H, SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ; Z is H or R^4 ; X is SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ; R^1 , R^2 , R^3 and R^4 are independently H or straight or branched chain or cyclic lower alkyl; n is 0, 1 or 2; wherein (peptide) is a peptide of 2 to about 10 amino acids; and: (1) where B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), X is SH and n is 1 or 2; (2)

where X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), B is SH and n is 1 or 2; (3) where B is H or R^4 , A is $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC , X is SH and n is 0 or 1; (4) where A is H or R^4 , then where B is SH, X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide); (5) where X is H or R^4 , A is $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC and B is SH; (6) where Z is methyl, X is methyl, A is $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC and B is SH and n is 0; and (7) where B is SH and X is SH, n is not 0; and wherein the thiol moiety is in the reduced form and wherein (amino acid) is any primary α - or β -amino acid not containing a thiol group.

In particular embodiments of this aspect of the invention, the radiolabel-complexing moiety has a formula that is:

Ila. $-(\text{amino acid})^1-(\text{amino acid})^2-\{A-CZ(B)-\{C(R^1R^2)\}_n-X\}$,

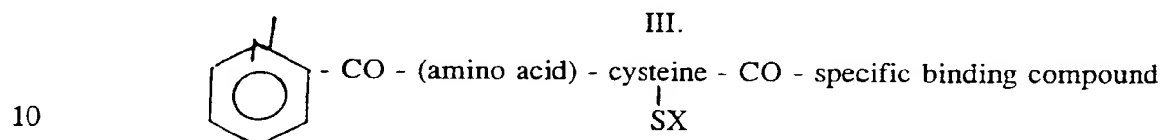
Ilb. $-\{A-CZ(B)-\{C(R^1R^2)\}_n-X\}-(\text{amino acid})^1-(\text{amino acid})^2$,

Ilc. $-(\text{a primary } \alpha, \omega\text{- or } \beta, \omega\text{-diamino acid})-(\text{amino acid})^1-\{A-CZ(B)-\{C(R^1R^2)\}_n-X\}$, or

Ild. $-\{A-CZ(B)-\{C(R^1R^2)\}_n-X\}-(\text{amino acid})^1-(\text{a primary } \alpha, \beta\text{- or } \beta, \gamma\text{-diamino acid})$ wherein $(\text{amino acid})^1$ and $(\text{amino acid})^2$ are each independently any naturally-occurring, modified, substituted or altered α - or β -amino acid not containing a thiol group; A is H, $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC or R^4 ; B is H, SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ; Z is H or R^4 ; X is SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ; R^1 , R^2 , R^3 and R^4 are independently H or straight or branched chain or cyclic lower alkyl; n is an integer that is either 0, 1 or 2; (peptide) is a peptide of 2 to about 10 amino acids; and: (1) where B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), X is SH and n is 1 or 2; (2) where X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), B is SH and n is 1 or 2; (3) where B is H or R^4 , A is $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC , X is SH and n is 0 or 1; (4) where A is H or R^4 , then where B is SH, X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide); (5) where X is H or R^4 , A is $HOOC$, H_2NOC , (amino acid or peptide)- $NHOC$, (amino acid or peptide)- OOC and B is SH; (6) where Z is methyl, X is methyl, A is $HOOC$, H_2NOC , (amino acid or peptide)-

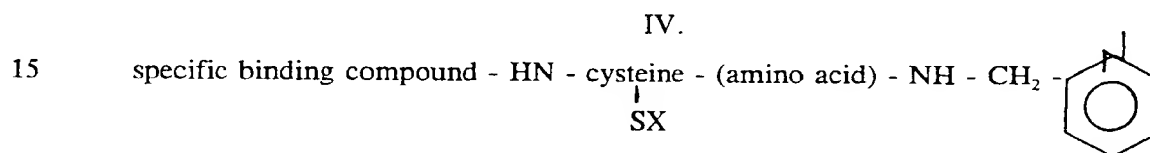
NHOC, (amino acid or peptide)-OOC and B is SH and n is 0; and (7) where B is SH and X is SH, n is not 0; and wherein the thiol group is in the reduced form.

In another embodiment, the invention provides a reagent for preparing a thrombus imaging agent that is capable of being radiolabeled for imaging thrombi within a mammalian body, comprising a specific binding compound that specifically binds to the platelet GPIIb/IIIa receptor, and that is covalently linked to a radiolabel-complexing moiety of formula:



(for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolinic acid (Pic)-based moieties);

or

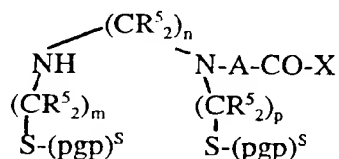


(for purposes of this invention, radiolabel-binding moieties having this structure will be referred to as picolylamine (Pica)-based moieties); wherein X is H or a protecting group; (amino acid) is any primary α - or β -amino acid not containing a thiol group; the radiolabel-complexing moiety is covalently linked to the specific binding compound and the complex of the radiolabel-complexing moiety and the radiolabel is electrically neutral. In a preferred embodiment, the amino acid is glycine and X is an acetamidomethyl protecting group. In additional preferred embodiments, the specific binding compound is covalently linked to the radiolabel-complexing moiety via an amino acid, most preferably glycine.

Yet another embodiment of the invention provides a reagent for preparing a thrombus imaging agent that is capable of being radiolabeled for imaging thrombi within a mammalian body, comprising a specific binding compound that specifically binds to the platelet GPIIb/IIIa receptor, and that is covalently linked to a radiolabel-complexing moiety that is a bisamino bisthiol radiolabel-complexing moiety. The bisamino bisthiol moiety in this embodiment of the invention has a formula selected

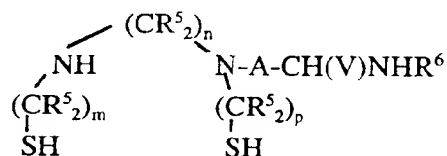
from the group consisting of:

V.



wherein each R^5 can be independently H, CH_3 or C_2H_5 ; each $(\text{pgp})^s$ can be independently a thiol protecting group or H; m, n and p are independently 2 or 3; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; and X is a specific binding compound; and

VI.



wherein each R^5 is independently H, lower alkyl having 1 to 6 carbon atoms, phenyl, or phenyl substituted with lower alkyl or lower alkoxy; m, n and p are independently 1 or 2; A is linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or substituted derivatives thereof; V is H or CO-(amino acid or peptide); R^6 is H, (amino acid) or peptide or a specific binding compound; provided that when V is H, R^6 is amino acid or peptide or a specific binding compound and when R^6 is H, V is amino acid or peptide or a specific binding compound, wherein (amino acid) is any primary α - or β -amino acid not containing a thiol group. (For purposes of this invention, radiolabel-binding moieties having these structures will be referred to as "BAT" moieties). In a preferred embodiment, the specific binding compound is covalently linked to the radiolabel-complexing moiety *via* an amino acid, most preferably glycine.

In preferred embodiments of the aforementioned aspects of this invention, the specific binding compound is a peptide is comprised of between 4 and 100 amino acids. The most preferred embodiment of the radiolabel is technetium-99m.

The reagents of the invention may be formed wherein the specific binding compounds or the radiolabel-complexing moieties are covalently linked to a polyvalent linking moiety. Polyvalent linking moieties of the invention are

comprised of at least 2 identical linker functional groups capable of covalently bonding to specific binding compounds or radiolabel-complexing moieties. Preferred linker functional groups are primary or secondary amines, hydroxyl groups, carboxylic acid groups or thiol-reactive groups. In preferred embodiments, the

5 polyvalent linking moieties are comprised of *bis*-succinimidylmethylether (BSME), 4-(2,2-dimethylacetyl)benzoic acid (DMAB), *tris*(succinimidylethyl)amine (TSEA), *tris*(acetamidoethyl)amine, *bis*-(acetamidoethyl)ether, *bis*-(acetamidomethyl)ether, *N*-{2-(*N'*,*N'*-*bis*(2-succinimidoethyl)aminoethyl)}-*N'*,*N'*-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide (BAT-BS), α,ϵ -*bis*acetyllysine, lysine and 1,8-*bis*-acetamido-3,6-dioxa-octane.

10

The invention also comprises scintigraphic imaging agents that are complexes of the reagents of the invention with Tc 99m, ¹¹¹In or ⁶⁸Ga, most preferably Tc-99m and methods for radiolabeling the reagents of the invention to provide such scintigraphic imaging agents. Tc-99m radiolabeled complexes provided by the

15 invention are formed by reacting the reagents of the invention with Tc-99m in the presence of a reducing agent. Preferred reducing agents include but are not limited to dithionite ion, stannous ion and ferrous ion. Complexes of the invention are also formed by labeling the reagents of the invention with Tc-99m by ligand exchange of a prereduced Tc-99m complex as provided herein.

20 The invention also provides kits for preparing scintigraphic imaging agents that are the reagents of the invention radiolabeled with Tc-99m. Kits for labeling the reagents provided by the invention with Tc-99m are comprised of a sealed vial containing a predetermined quantity of a reagent of the invention and a sufficient amount of reducing agent to label the reagent with Tc-99m.

25 This invention provides methods for preparing peptide reagents of the invention by chemical synthesis *in vitro*. In a preferred embodiment, peptides are synthesized by solid phase peptide synthesis.

This invention provides methods for using scintigraphic imaging agents that are Tc-99m labeled reagents for imaging thrombi within a mammalian body by

30 obtaining *in vivo* gamma scintigraphic images. These methods comprise administering an effective diagnostic amount of Tc-99m labeled reagents of the invention and detecting the gamma radiation emitted by the Tc-99m label localized

at the thrombus site within the mammalian body.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates scintigraphic imaging of deep-vein thrombi in the thigh in human patients using a Tc-99m radiolabel peptide reagent of the invention.

Figure 2 illustrates scintigraphic imaging of deep-vein thrombi in the calf in human patients using a Tc-99m radiolabel peptide reagent of the invention.

10

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides reagents, including peptide reagents, for preparing radiolabeled thrombus imaging agents for imaging a thrombus within a mammalian body. The reagents provided by the invention comprise a radiolabel binding moiety covalently linked to a specific binding compound that binds a platelet receptor that is the platelet GPIIb/IIIa receptor and is capable of inhibiting human platelet aggregation in platelet-rich plasma by 50% when present at a concentration of no more than $1\mu\text{M}$ (*i.e.*, $\text{IC}_{50} = 1\mu\text{M}$). For purposes of the invention, the term thrombus imaging reagent will refer to embodiments of the invention comprising a specific binding compound covalently linked to a radiolabel-complexing moiety and radiolabeled, preferably with Tc-99m, ^{111}In or ^{68}Ga , most preferably with Tc-99m.

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We have previously found that, for optimal imaging, a reagent as disclosed herein must be capable of binding to platelet glycoprotein IIb/IIIa receptor with sufficient affinity that it inhibits adenosine diphosphate (ADP)-induced platelet aggregation in a standard assay (*see* Example 3) when present at concentrations up to $0.3\mu\text{M}$ ($\text{IC}_{50} \leq 0.3\mu\text{M}$). This invention was disclosed in U.S. Patent Applications Serial No. 08/044,825 and International Patent Application Serial No. PCT/US94/03878, the disclosures of both incorporated by reference in their entireties).

We have now found that advantageously high-quality *in vivo* scintigraphic images may be obtained using a radiolabeled scintigraphic imaging agent as disclosed

herein having an $IC_{50} \leq 1\mu M$.

5 Labeling with Tc-99m is an advantage of the present invention because the nuclear and radioactive properties of this isotope make it an ideal scintigraphic imaging agent. This isotope has a single photon energy of 140 keV and a radioactive half-life of about 6 hours, and is readily available from a ^{99}Mo - ^{99m}Tc generator. Another advantage of the present invention is that none of the preferred radionuclides (Tc-99m, Ga-67, In-111) are toxic, in contrast to other radionuclides known in the art (*for example*, ^{125}I).

10 In the Tc-99m complexing moieties and compounds covalently linked to such moieties that contain a thiol covalently linked to a thiol protecting group $\{(pgp)^S\}$ provided by the invention, the thiol-protecting groups may be the same or different and may be but are not limited to:

- CH₂-aryl (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- CH-(aryl)₂, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- C-(aryl)₃, (aryl is phenyl or alkyl or alkyloxy substituted phenyl);
- CH₂-(4-methoxyphenyl);
- 5 -CH-(4-pyridyl)(phenyl)₂;
- C(CH₃)₃
- 9-phenylfluorenyl;
- CH₂NHCOR (R is unsubstituted or substituted alkyl or aryl);
- CH₂-NHCOOR (R is unsubstituted or substituted alkyl or aryl);
- 10 -CONHR (R is unsubstituted or substituted alkyl or aryl);
- CH₂-S-CH₂-phenyl

Preferred protecting groups have the formula -CH₂-NHCOR wherein R is a lower alkyl having 1 and 8 carbon atoms, phenyl or phenyl-substituted with lower alkyl, hydroxyl, lower alkoxy, carboxy, or lower alkoxy carbonyl. The most preferred protecting group is an acetamidomethyl group.

Each specific-binding peptide-containing embodiment of the invention is comprised of a sequence of amino acids. The term amino acid as used in this invention is intended to include all L- and D-, primary α - or β -amino acids, naturally occurring and otherwise. Specific-binding peptides provided by the invention include but are not limited to peptides having the following sequences (the amino acids in the following peptides are L-amino acids except where otherwise indicated):

CH₃CO.Y_D.Apc.GDCGGG
CH₃CO.Y_D.Apc.GDCKG
CH₃CO.Y_D.Apc.GDCGG
 25 CH₃CO.Y_D.Apc.GDC
CH₃CO.Y_D.Apc.GDCK
CH₃CO.Y_D.Amp.GDC
CH₃CO.Y_D.Amp.GDCK
 and O-(4-piperidinyl)butyl tyrosine.

Specific-binding peptides of the present invention can be chemically synthesized *in vitro*. Peptides of the present invention can generally advantageously be prepared on an peptide synthesizer. The peptides of this invention can be synthesized wherein the radiolabel-binding moiety is covalently linked to the peptide during chemical synthesis *in vitro*, using techniques well known to those with skill in the art. Such peptides covalently linked to the radiolabel-binding moiety during

synthesis are advantageous because specific sites of covalent linkage can be determined.

Radiolabel binding moieties of the invention may be introduced into the target specific peptide during peptide synthesis. For embodiments comprising picolinic acid
5 {(Pic-); *e.g.*, Pic-Gly-Cys(protecting group)-}, the radiolabel-binding moiety can be synthesized as the last (*i.e.*, amino-terminal) residue in the synthesis. In addition, the picolinic acid-containing radiolabel-binding moiety may be covalently linked to the ϵ -amino group of lysine to give, for example, αN (Fmoc)-Lys- ϵN {Pic-Gly-Cys(protecting group)}, which may be incorporated at any position in the peptide
10 chain. This sequence is particularly advantageous as it affords an easy mode of incorporation into the target binding peptide.

Similarly, the picolylamine (Pica)-containing radiolabel-binding moiety {Cys(protecting group)-Gly-Pica} can be prepared during peptide synthesis by including the sequence {-Cys(protecting group)-Gly-} at the carboxyl terminus of the
15 peptide chain. Following cleavage of the peptide from the resin the carboxyl terminus of the peptide is activated and coupled to picolylamine. This synthetic route requires that reactive side-chain functionalities remain masked (protected) and do not react during the conjugation of the picolylamine.

Examples of small synthetic peptides containing the Pic-Gly-Cys- and -Cys-Gly-Pica chelators are provided in the Examples hereinbelow. This invention provides for the incorporation of these chelators into virtually any peptide capable of specifically binding to a thrombus *in vivo*, resulting in a radiolabeled peptide having Tc-99m held as neutral complex.

This invention also provides specific-binding small synthetic peptides which
25 incorporate bisamine bisthiol (BAT) chelators which may be labeled with Tc-99m. This invention provides for the incorporation of these chelators into virtually any peptide capable of specifically binding to a thrombus *in vivo*, resulting in a radiolabeled peptide having Tc-99m held as neutral complex. An example of a small synthetic peptide containing a BAT chelator as radiolabel-binding moiety is provided
30 in the Examples hereinbelow.

In forming a complex of radioactive technetium with the reagents of this invention, the technetium complex, preferably a salt of Tc-99m pertechnetate, is

reacted with the reagent in the presence of a reducing agent. Preferred reducing agents are dithionite, stannous and ferrous ions; the most preferred reducing agent is stannous chloride. Means for preparing such complexes are conveniently provided in a kit form comprising a sealed vial containing a predetermined quantity of a reagent of the invention to be labeled and a sufficient amount of reducing agent to label the reagent with Tc-99m. Alternatively, the complex may be formed by reacting a reagent of this invention with a pre-formed labile complex of technetium and another compound known as a transfer ligand. This process is known as ligand exchange and is well known to those skilled in the art. The labile complex may be formed using such transfer ligands as tartrate, citrate, gluconate or mannitol, for example. Among the Tc-99m pertechnetate salts useful with the present invention are included the alkali metal salts such as the sodium salt, or ammonium salts or lower alkyl ammonium salts.

In a preferred embodiment of the invention, a kit for preparing technetium-labeled reagents is provided. An appropriate amount of the reagent is introduced into a vial containing a reducing agent, such as stannous chloride, in an amount sufficient to label the reagent with Tc-99m. An appropriate amount of a transfer ligand as described (such as tartrate, citrate, gluconate or mannitol, for example) can also be included. The kit may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, buffers, preservatives and the like. The components of the kit may be in liquid, frozen or dry form. In a preferred embodiment, kit components are provided in lyophilized form.

Radiolabeled thrombus imaging reagents according to the present invention may be prepared by the addition of an appropriate amount of Tc-99m or Tc-99m complex into the vials and reaction under conditions described in Example 4 hereinbelow.

Radioactively-labeled scintigraphic imaging agents provided by the present invention are provided having a suitable amount of radioactivity. In forming Tc-99m radioactive complexes, it is generally preferred to form radioactive complexes in solutions containing radioactivity at concentrations of from about 0.01 millicurie (mCi) to 100 mCi per mL.

The thrombus imaging reagents provided by the present invention can be used for visualizing thrombi in a mammalian body when Tc-99m labeled. In accordance with this invention, the Tc-99m labeled reagents are administered in a single unit injectable dose. The Tc-99m labeled reagents provided by the invention may be administered intravenously in any conventional medium for intravenous injection such as an aqueous saline medium, or in blood plasma medium. Generally, the unit dose to be administered has a radioactivity of about 0.01 mCi to about 100 mCi, preferably 1 mCi to 20 mCi. The solution to be injected at unit dosage is from about 0.01 mL to about 10 mL. After intravenous administration, imaging of the thrombus *in vivo* can take place in a matter of a few minutes. However, imaging can take place, if desired, in hours or even longer, after the radiolabeled peptide is injected into a patient. In most instances, a sufficient amount of the administered dose will accumulate in the area to be imaged within about 0.1 of an hour to permit the taking of scintiphotos. Any conventional method of scintigraphic imaging for diagnostic purposes can be utilized in accordance with this invention.

It will also be recognized by those having skill in the relevant arts that thrombi are commonly found at sites of atherosclerotic plaque; that integrin receptors that may bind to the scintigraphic imaging agents of the invention may be found in certain tumors; and that such integrin receptors are involved in cell adhesion processes that accompany or initiate leukocyte localization at sites of infection. Therefore it will be recognized that the scintigraphic imaging agents of this invention have additional utility as imaging agents for imaging sites in which the GPIIb/IIIa receptor is expressed, including atherosclerotic plaques, tumors and sites of infection.

The methods for making and labeling these compounds are more fully illustrated in the following Examples. These Examples illustrate certain aspects of the above-described method and advantageous results. These Examples are shown by way of illustration and not by way of limitation.

EXAMPLE 1

Solid Phase Peptide Synthesis

5 Solid phase peptide synthesis (SPPS) was carried out on a 0.25 millimole (mmole) scale using an Applied Biosystems Model 431A Peptide Synthesizer and using 9-fluorenylmethyloxycarbonyl (Fmoc) amino-terminus protection, coupling with dicyclohexylcarbodiimide/hydroxybenzotriazole or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate/hydroxybenzotriazole (HBTU/HOBT), and
10 using *p*-hydroxymethylphenoxymethylpolystyrene (HMP) resin for carboxyl-terminus acids or Rink amide resin for carboxyl-terminus amides. Resin-bound products were routinely cleaved using a solution comprised of trifluoroacetic acid or 50/50 trifluoroacetic acid/dichloromethane, optionally containing water, thioanisole, ethanedithiol, and triethylsilane, prepared in ratios of 100 : 5 : 5 : 2.5 : 2 for 1.5 -
15 3 h at room temperature.

Where appropriate, N-terminal acetyl groups were introduced by treating the free N-terminal amino peptide bound to the resin with 20% v/v acetic anhydride in NMP (*N*-methylpyrrolidinone) for 30 min. For preparing branched-chain peptide reagents involving peptide chain synthesis from both the α - and ϵ -amines of lysine,
20 *N* α (Fmoc)*N* ϵ (Fmoc)-lysine was used during SPPS. Where appropriate, 2-chloroacetyl and 2-bromoacetyl groups were introduced either by using the appropriate 2-halo-acetic acid as the last residue to be coupled during SPPS or by treating the N-terminus free amino peptide bound to the resin with either 2-halo-acetic acid/ diisopropylcarbodiimide/ *N*-hydroxysuccinimide in NMP or 2-halo-acetic
25 anhydride/ diisopropylethylamine in NMP. Where appropriate, HPLC-purified 2-haloacetylated peptides were cyclized by stirring in a 0.1 - 1.0 mg/mL solution at pH8 optionally containing phosphate, bicarbonate or 0.5-1.0 mM EDTA for 0.5 - 48 hours, followed by acidification with acetic acid, lyophilization and HPLC purification. Where appropriate, Cys-Cys disulfide bond cyclizations were
30 performed by treating the precursor cysteine-free thiol peptides at 0.1mg/mL in pH 7 buffer with aliquots of 0.006 M $K_3Fe(CN)_6$ until a stable yellow color persisted. The excess oxidant was reduced with excess cysteine, the mixture was lyophilized and then purified by HPLC.

Where appropriate, peptide thiol-chloroacetyl derived sulfides were prepared

by reacting single thiol-containing peptides at a concentration of 2 to 50 mg/mL in water and acetonitrile or THF or DMF at pH 10 with the appropriate number (*e.g.*, 0.5 molar equivalents for preparing dimers and 0.33 molar equivalents for preparing trimers) of the chloroacetyl polyvalent linker moiety for 0.5 to 24 hours. The solution was then neutralized with acetic acid, evaporated to dryness, and, if necessary, deprotected using 10 mL TFA and scavengers such as 0.2 mL triethylsilane for 30 to 90 minutes. The solution was concentrated and the product was precipitated with ether. Products were purified by preparative HPLC.

Where appropriate, BSME adducts were prepared by reacting single thiol-containing peptides (5 to 50 mg/mL in 50 mM sodium phosphate buffer, pH 7 to 8) with 0.5 molar equivalents of BMME (*bis*-maleimidomethylether) pre-dissolved in acetonitrile at room temperature for approximately 1-18 hours. The solution was concentrated and the product was purified by HPLC.

Where appropriate, TSEA adducts were prepared by reacting single thiol-containing peptide (at concentrations of 10 to 100 mg/mL peptide in DMF, or 5 to 50 mg/mL peptide in 50mM sodium phosphate (pH 8)/ acetonitrile or THF) with 0.33 molar equivalents of TMEA (*tris*(2-maleimidoethyl)amine; as disclosed in U.S. Serial No. 08/044,825, incorporated by reference) pre-dissolved in acetonitrile or DMF, with or without 1 molar equivalent of triethanolamine, at room temperature for approximately 1-18h. Such reaction mixtures containing adducts were concentrated and the adducts were then purified using HPLC.

Where appropriate, BAT-BS adducts were prepared by reacting single thiol-containing peptide (at concentrations of 2 to 50 mg/mL peptide in 50mM sodium phosphate (pH 8)/ acetonitrile or THF) with 0.5 molar equivalents of BAT-BM (*N*-{2-(*N'*,*N'*-*bis*(2-maleimido-ethyl)aminoethyl)}-*N*⁶-(*t*-butoxycarbonyl)-*N*⁶,*N*⁶-*bis*(2-methyl-2-triphenylmethylthiopropyl)-6,9-diazanonanamide; as disclosed in U.S. Serial No. 08/044,825, incorporated by reference) pre-dissolved in acetonitrile or THF, at room temperature for approximately 1-18h. The solution was then evaporated to dryness and (BAT-BS)-peptide conjugates deprotected by treatment with 10mL TFA and 0.2mL triethylsilane for 1h. The solution was concentrated, the product adducts precipitated with ether, and then purified by HPLC.

Crude peptides were purified by preparative high pressure liquid

chromatography (HPLC) using a Waters Delta Pak C18 column and gradient elution using 0.1% trifluoroacetic acid (TFA) in water modified with acetonitrile. Acetonitrile was evaporated from the eluted fractions which were then lyophilized. The identity of each product was confirmed by fast atom bombardment mass spectroscopy (FABMS) or electrospray mass spectroscopy (ESMS).

EXAMPLE 2

A General Method for Radiolabeling with Tc-99m

A 0.1 mg sample of a peptide reagent prepared as in Example 2 was dissolved in 0.1 mL of water, 50 mM potassium phosphate buffer, 0.1 M bicarbonate buffer or 10% hydroxypropylcyclo-dextrin (HPCD), each buffer at pH of 5-10. Tc-99m gluceptate was prepared by reconstituting a Glucoscan vial (E.I. DuPont de Nemours, Inc., Wilmington, DE) with 1.0mL of Tc-99m sodium pertechnetate containing up to 200mCi and allowed to stand for 15 minutes at room temperature. 25 μ L of Tc-99m gluceptate was then added to the peptide and the reaction allowed to proceed at room temperature or at 100°C for 5-30 min and then filtered through a 0.2 μ m filter.

The Tc-99m labeled peptide purity was determined by HPLC using the following conditions: a Waters DeltaPure RP-18, 5 μ , 150mm x 3.9mm analytical column was loaded with each radiolabeled peptide and the peptides eluted at a solvent flow rate equal to 1 mL/min. Gradient elution was performed beginning with 10% solvent A (0.1% CF₃COOH/H₂O) to 40% solvent B₉₀ (0.1% CF₃COOH/90% CH₃CN/H₂O) over the course of 20 min.

The Tc-99m labeled peptide purity was determined by HPLC using the conditions described in the Footnotes in Table I. Radioactive components were detected by an in-line radiometric detector linked to an integrating recorder. Tc-99m gluceptate and Tc-99m sodium pertechnetate elute between 1 and 4 minutes under these conditions, whereas the Tc-99m labeled peptide eluted after a much greater amount of time.

The following Table illustrates successful Tc-99m labeling of peptides prepared according to Example 1 using the method described herein.

TABLE I

Peptides	FAB/MS MH ⁺	Radiochemical Yield(%) [*]	HPLC R _T (min) ^{**}
<u>CH₂CO</u> .Y _D . <u>RGDCC</u> _{Acm} .GC _{Acm} .amide ^b	1057	97 ²	10.0, 10.4, 10.6 ²
<u>CH₂CO</u> .Y _D . <u>RGDCGGC</u> _{Acm} .GC _{Acm} .amide	1171	99 ²	13.5 ²
<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGGC</u> _{Acm} .GC _{Acm} .amide	1233	100 ⁴	17.1, 18.1 ²
GRGDVRGDFKC _{Acm} .GC _{Acm} .amide	1510	97 ²	16.2, 16.8 ²
GRGDVRGDFC _{Acm} .GC _{Acm} .amide	1382	94 ²	16.4 ²
<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGC</u> _{Acm} .GC _{Acm} .GGF _D .PRPG.NH ₂	1845	90 ⁴	16.6, 16.9 ²
(<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₂ -BSME	3020 ^a	98 ⁴	9.3 ²
(<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₃ -TSEA	4596	99 ⁴	9.2, 11.6 ⁵
(<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₂ -(BAT-BS)	3409 ^a	98 ³	10.3 ⁵
C _{Acm} .GC _{Acm} .RRRRRRRRRGDV	2100	100 ²	2.4 ^{3***}
(<u>CH₂CO</u> .Y _D . <u>Apc.GDCCKGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₂ -BSME	3163 ^a	98 ⁴	9.6 ⁵
(<u>CH₂CO</u> .Y _D . <u>Amp.GDCGGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₂ -(CH ₂ CO) ₂ K(Nε-K)GCamide	3357 ^a	99 ⁸	4.6 ⁶
(<u>CH₂CO</u> .Y _D . <u>Amp.GDCCKGCGamide</u>) ₂ -(CH ₂ CO) ₂ K(Nε-K)GCamide	2573 ^a	99 ⁸	4.8 ⁶
(<u>CH₂CO</u> .Y _D . <u>Apc.GDCGGC</u> _{Acm} .GC _{Acm} .GGC.amide) ₂ -(CH ₂ CO) ₂ K(Nε-K)GCamide	3298 ^a	96 ³	12.0 ⁴

* Superscripts refer to the following labeling conditions:

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1. The peptide was dissolved in 50 mM potassium phosphate buffer (pH 7.4) and labeled at room temperature.
2. The peptide was dissolved in 50 mM potassium phosphate buffer (pH 7.4) and labeled at 100°C.
- 5 3. The peptide was dissolved in water and labeled at room temperature.
4. The peptide was dissolved in water and labeled at 100°C.
5. The peptide was dissolved in 50 mM potassium phosphate buffer (pH 6.0) and labeled at 100°C.
6. The peptide was dissolved in 50 mM potassium phosphate buffer (pH 5.0) and labeled at room temperature.
- 10 7. The peptide was dissolved in a 50:50 mixture of ethanol/water and labeled at 100°C.
8. The peptide was dissolved in 0.9% sodium chloride solution and labeled at room temperature.

15

** HPLC methods (indicated by superscript after R_T):

general: solvent A = 0.1% CF₃COOH/H₂O
 solvent B₇₀ = 0.1% CF₃COOH/70% CH₃CN/H₂O
 20 solvent B₉₀ = 0.1% CF₃COOH/90% CH₃CN/H₂O
 solvent flow rate = 1 mL/min

Vydak column = Vydak 218TP54 RP-18, 5μm, 220mm x 4.6mm analytical column with guard column

25 Brownlee column = Brownlee Spheri-5 RP-18, 5μm, 220mm x 4.6mm column

Waters column = Waters Delta-Pak C18, 5μm, 150mm x 3.9mm column

Waters column 2 = Waters Nova-Pak C18, 5μm, 100mm x 8mm radial compression column

30	Method 1:	Brownlee column	100% A to 100% B ₇₀ in 10 min
	Method 2:	Vydak column	100% A to 100% B ₉₀ in 10 min
	Method 3:	Vydak column	100% A to 100% B ₇₀ in 10 min
	Method 4:	Waters column	100% A to 100% B ₉₀ in 20 min
	Method 5:	Waters column	100% A to 100% B ₉₀ in 10 min
35	Method 6:	Waters 2 column	100% A to 100% B ₉₀ in 10 min

*** Confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis

40 Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry* (2d. ed.), 1988 (MacMillen Publishing: New York) p.33; underlining indicates the formation of a thiol linkage between the linked amino acids of derivative groups; peptides are linked to BSH, ETAC, BSME, TSEA, (BAT-BS) or (CH₂CO)-containing linkers *via* the free thiol moiety of the unprotected cysteine residue (C) in each peptide; Ac = acetyl; Bz = benzoyl; Pic = picolinoyl
 45 (pyridine-2-carbonyl); Ac_m = acetamidomethyl; Mob = 4-methoxybenzyl; Apc = L-(S-(3-aminopropyl)cysteine); Hly = homolysine; F_D = D-phenylalanine; Y_D = D-tyrosine; ma = 2-mercaptoacetic acid; mmp = 2-mercapto-2-methylpropionic acid; BAT = N⁶,N⁹-bis(2-mercapto-2-methylpropyl)-6,9-diazanonanoic acid; ETAC = 4-

(O-CH₂CO-Gly-Gly-Cys.amide)acetophenone; BAT-BS = N-{2-N',N'-bis(2-succinimidoethyl) aminoethyl}-N⁶,N⁹-bis(2-mercapto-2-methylpropyl)-6,9-diazanonanamide; BSME = bis-succinimidomethylether; TSEA = tris-(2-succinimidoethyl)amine; NES = N-ethylsuccinimide; BSH = 1,6-bis-succinimidohexane; Amp = 4-amidinophenylalanine

^a = confirmed by electrospray mass spectrometry (ESMS)

EXAMPLE 3

Platelet Aggregation Inhibition Assays

Platelet aggregation studies were performed essentially as described by Zucker (1989, *Methods in Enzymol.* 169: 117-133). Briefly, platelet aggregation was assayed with or without putative platelet aggregation inhibitory compounds using fresh human platelet-rich plasma, comprising 300,000 platelets per microlitre. Platelet aggregation was induced by the addition of a solution of adenosine diphosphate to a final concentration of 10 to 15 micromolar, and the extent of platelet aggregation monitored using a Bio/Data aggregometer (Bio/Data Corp., Horsham, PA). The concentrations of platelet aggregation inhibitory compounds used were varied from 0.1 to 500 µg/mL. The concentration of inhibitor that reduced the extent of platelet aggregation by 50% (defined as the IC₅₀) was determined from plots of inhibitor concentration *versus* extent of platelet aggregation. An inhibition curve for peptide RGDS was determined for each batch of platelets tested.

The results of these experiments are shown in Table II. In Table II, the compounds tested are as follows (RGDS is given as a positive control):

P47	=	AcSYGRGDVRGDFKC _{Acm} GC _{Acm}
P97	=	GRGDVRGDFKC _{Acm} GC _{Acm} amide
P32	=	C _{Acm} GC _{Acm} RRRRRRRRRGDV
P143	=	CH ₃ CO-Y _D RGDCGGC _{Acm} GC _{Acm} amide
P245	=	CH ₃ CO-Y _D Apc.GDCGGC _{Acm} GC _{Acm} GGF _D PRPGamide
P63	=	AcSYGRGDVRGDFKCTCCA
P98	=	GRDGVRGDFC _{Acm} GC _{Acm} amide
P81	=	CH ₃ CO-Y _D RGDCC _{Acm} GC _{Acm} amide
P154	=	CH ₃ CO-Y _D ApcGDCGGGC _{Acm} GC _{Acm} amide
P381	=	(CH ₃ CO-Y _D ApcGDCKGC _{Acm} GC _{Acm} GGC-amide) ₂ -BSME

- P317 = $(\text{CH}_2\text{CO-Y}_D\text{ApcGDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGC-amide})_3\text{-TSEA}$
 P246 = $\text{CH}_2\text{CO-Y}_D\text{ApcGDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGC-amide}$
 P357 = $(\text{CH}_2\text{CO-Y}_D\text{ApcGDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGC-amide})_2\text{-(BAT-BS)}$
 P667 = $(\text{CH}_2\text{COY}_D\text{-Apc-GDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGCamide})_2(\text{CH}_2\text{CO})_2\text{K(N}\epsilon\text{-K)GCamide}$
 5 P747 = $(\text{CH}_2\text{COY}_D\text{-Amp-GDCGGC}_{\text{Acm}}\text{GC}_{\text{Acm}}\text{GGCamide})_2(\text{CH}_2\text{CO})_2\text{K(N}\epsilon\text{-K)GCamide}$
 P748 = $(\text{CH}_2\text{COY}_D\text{-Amp-GDCKGCGamide})_2(\text{CH}_2\text{CO})_2\text{K(N}\epsilon\text{-K)GCamide}$

(Single-letter abbreviations for amino acids can be found in G. Zubay, *Biochemistry*
 10 (2d. ed.), 1988 (MacMillen Publishing: New York) p.33; Ac = acetyl; Acm =
 acetamidomethyl; Apc = L-(S-(3-aminopropyl)cysteine); Y_D = D-tyrosine; BSME
 = *bis*-succinimidylmethylether; TSEA = *tris*(succinimidylethyl)amine; (BAT-BS) =
 N-{2-(N',N'-bis(2-succinimidoethyl) aminoethyl)}-N⁶,N⁹,-bis(2-methyl-2-
 15 mercaptopropyl)-6,9-diazanonanamide; peptides are linked to BSME. TSEA, (BAT-
 BS) or (CH₂CO)-containing linkers *via* the free thiol moiety of the unprotected
 cysteine residue (C) in each peptide). (...)K represents a covalent bond between the
 moiety in parenthesis and each of the amino groups (*i.e.*, the α-amino and the
 sidechain amine) of lysine. (Nε-K) represents covalent linkage at the ε amine rather
 than at the usual α amino group of the lysine residue.

TABLE II

Peptides	IC ₅₀ (μ M)**	Clot/Blood*
P357	0.079	6.3 \pm 3.4 ⁵
P667	0.081	5.9, 5.0 ²
P682	0.130	4.0 ¹
P317	0.036	3.8 \pm 2.2 ³
P381	0.035	2.5
P154	0.30	2.0 \pm 0.5 ³
P246	0.85	4.4 \pm 1.8
P143	1.3	1.4
P97	8	1.0
P98	15	1.7
P63	19	1.7
P47	23	1.0
P81	25	1.8 \pm 0.6 ³
P32	26	1.2 \pm 0.2 ⁴

¹n = 1; ²n = 2; ³n = 3; ⁴n = 4; ⁵n = 9

- 20 * ratio of (% injected dose/g in a femoral vein thrombus)/ (% injected dose/g in blood) at approximately 4h post-injection of each Tc-99m labeled reagent in a canine model of DVT
- 25 ** concentration of reagent that inhibits by 50% the aggregation of human platelets in platelet-rich plasma induced to aggregate by the addition of adenosine diphosphate (ADP)

These results demonstrate that the compounds having an IC_{50} less than or equal to about $1\mu M$ show greater efficacy as scintigraphic imaging agents than compounds having an IC_{50} greater than about $1\mu M$.

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EXAMPLE 4

Scintigraphic Imaging of Deep-Vein Thrombi in Humans *in vivo*

A series of experiments comprising a pilot human clinical study of one embodiment of the scintigraphic imaging agents of the invention, designated P280, having the chemical structure:

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were performed. These experiments were performed on 9 human patients (6 males, 3 females), ages 30 to 60 years and weighing 63 to 100 kg. Each of the patients presented clinically with the symptoms of deep-vein thrombosis, and the diagnosis was confirmed by physical work-up, ultrasonography and/or contrast venography.

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Each patient in the study was administered 10-22 mCi Tc-99m labeled P280 comprising approximately 0.25mg peptide, by intravenous injection. Scintigraphic imaging was then performed over four hours using a large field-of-view gamma camera equipped with a high-resolution collimator (photopeaked at 140 keV, with a $20\% \pm$ window). Gamma camera imaging was commenced simultaneously with injection. Anterior images followed by posterior images over the legs were acquired over the first hour, then at 2 and 4 hours post-injection.

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The results of these studies are shown in Figure 1, which shows thrombus localization in vessels of the lower thigh and Figure 2, which shows thrombus localization in vessels of the calf. Thrombi so localized are highlighted with an arrow in each Figure. In addition to rapidly and efficiently localizing sites of deep-vein thrombi, this scintigraphic imaging agent was found to clear rapidly from the bloodstream, resulting in less than about 10% of the injected dose remaining in the circulation 1 hour post-injection. Consistent with animal studies, at least 60-70% of the injected dose was found to be cleared by the kidneys. Thrombus visualization was evident as early as 15-30 min after injection, and remained visible for 4-24 hours post-injection. Thrombus visualization correlated well with the clinical diagnosis of deep-vein thrombosis made based on the aforementioned conventional

clinical criteria, thrombi being visualized in 8 of the 9 patients studied. The one patient in which a thrombus was not visualized presented with a clinically old thrombus (42 days), which was likely quiescent and hence no longer experiencing platelet turnover at its surface. Finally, no toxicity or other adverse effects of Tc-99m labeled P280 administration were observed in any of these patients.

These results demonstrate that the scintigraphic imaging agents of the invention represents a safe and effective diagnostic reagent for preparing clinically-effective scintigraphic imaging agents useful for clinical, *in vivo* use for visualizing deep-vein thrombi in humans.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

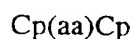
What is claimed is:

1. A reagent for preparing a thrombus imaging agent for imaging thrombi within a mammalian body, comprising a radiolabel complexing moiety covalently linked to a specific binding compound that is less than 10,000 daltons in molecular weight and that binds to a platelet receptor that is the platelet glycoprotein IIb/IIIa receptor, wherein the reagent is capable of inhibiting human platelet aggregation in platelet-rich plasma by 50% (IC_{50}) when present at a concentration of not greater than about $1\mu M$.

2. A reagent according to Claim 1 wherein the specific binding compound is a specific binding peptide comprising 4 to 100 amino acids.

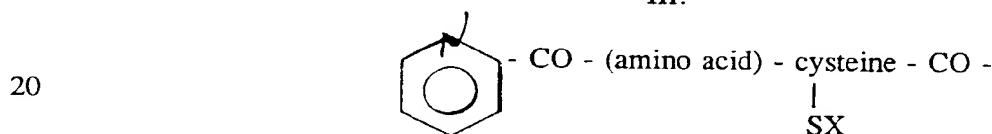
3. The reagent of Claim 1 wherein the radiolabel complexing moiety has a formula selected from the group consisting of:

I.

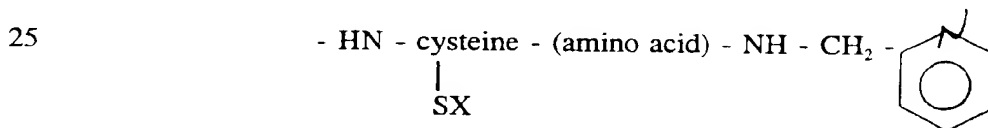


wherein Cp is a protected cysteine and (aa) is any primary α - or β -amino acid not containing a thiol group; and

III.

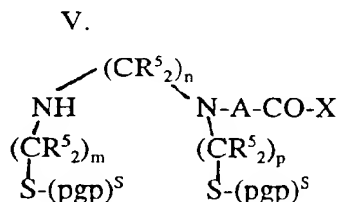


IV.



wherein X = H or a protecting group;

(amino acid) = any primary α - or β -amino acid not containing a thiol group;

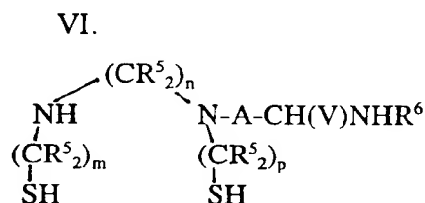


5

wherein each R^5 is independently H, CH_3 or C_2H_5 ;
 each $(\text{pgp})^s$ is independently a thiol protecting group or H;
 m, n and p are independently 2 or 3;
 A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or
 substituted derivatives thereof;
 X = a specific binding compound;

10

and



15

wherein each R^5 is independently H, lower alkyl having 1 to 6 carbon atoms,
 phenyl, or phenyl substituted with lower alkyl or lower alkoxy;
 m, n and p are independently 1 or 2;
 A = linear or cyclic lower alkyl, aryl, heterocyclyl, combinations or
 substituted derivatives thereof;
 V = H or -CO-specific binding compound;
 R^6 = H or specific binding compound;
 and wherein when V = H, R^6 = specific binding compound and when R^6 = H, V
 = -CO-specific binding compound.

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4. The reagent of Claim 1 wherein the specific binding compound and
 radiolabel complexing moiety are covalently linked through one or more amino acids.

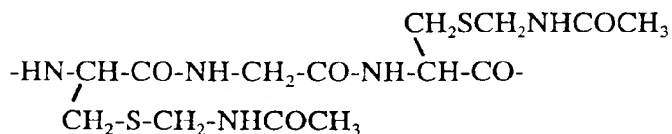
5. The reagent of Claim 4 wherein the protected cysteine of the
 radiolabel complexing moiety having formula I has a protecting group of the formula



wherein R is a lower alkyl having 1 to 6 carbon atoms, 2-,3-,4-pyridyl, phenyl, or
 phenyl substituted with lower alkyl, hydroxy, lower alkoxy, carboxy, or lower

alkoxycarbonyl.

6. The reagent of Claim 4 wherein the radiolabel complexing moiety of formula Cp(aa)Cp has the formula:



7. A scintigraphic imaging agent comprising the reagent according to Claim 1 wherein the radiolabel complexing moiety is bound to a radiolabel.

8. The reagent of Claim 7 wherein the radiolabel is technetium-99m, indium-111 or gallium-68.

9. The reagent of Claim 2 wherein the specific binding peptide is selected from the group consisting of peptides having the formula:

CH₃CO.Y_D.Apc.GDCGGG

CH₃CO.Y_D.Apc.GDCKG

CH₃CO.Y_D.Apc.GDCGG

CH₃CO.Y_D.Apc.GDC

CH₃CO.Y_D.Apc.GDCK

CH₃CO.Y_D.Amp.GDC

CH₃CO.Y_D.Amp.GDCK

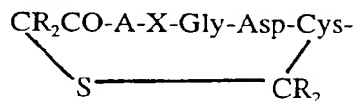
and O-(4-piperidiny)butyl tyrosine.

10. The reagent of Claim 1 wherein the reagent further comprises a polyvalent linking moiety covalently linked to a multiplicity of specific binding compounds and also covalently linked to a multiplicity of radiolabel complexing moieties to comprise a reagent for preparing a multimeric polyvalent scintigraphic imaging agent, wherein the molecular weight of the multimeric polyvalent scintigraphic imaging agent is less than about 20,000 daltons.

11. The reagent of Claim 10 wherein the polyvalent linking moiety is *bis*-succinimidylmethylether, 4-(2,2-dimethylacetyl)benzoic acid, *N*-{2-(*N',N'*-*bis*(2-succinimido-ethyl)aminoethyl)}-*N*⁶,*N*⁹-*bis*(2-methyl-2-mercaptopropyl)-6,9-diazanonanamide, *tris*(succinimidylethyl)amine, *tris*(acetamidoethyl)amine, *bis*-(acetamidoethyl)ether, *bis*-(acetamidomethyl)ether, α,ϵ -*bis*acetyllysine, lysine and 1,8-*bis*-acetamido-3,6-dioxo-octane, 1,2-*bis*(2-chloroacetamidoethoxy)ethane, or a derivative thereof.

12. A complex formed by reacting the reagent of Claim 2 with technetium-99m in the presence of a reducing agent.

13. The complex of Claim 12, wherein the reducing agent is selected from the group of a dithionite ion, a stannous ion, or a ferrous ion.
14. A complex formed by labeling the reagent of Claim 2 with technetium-99m by ligand exchange of a prereduced technetium-99m complex.
15. A kit for preparing a radiopharmaceutical preparation, said kit comprising sealed vial containing a predetermined quantity of the reagent of Claim 2 and a sufficient amount of reducing agent to label the reagent with technetium-99m.
16. Use of the reagent of Claim 2 for preparing a medicament for imaging thrombi within a mammalian body wherein the reagent is labeled with technetium-99m.
17. The process of preparing the reagent according to Claim 2 wherein the reagent is chemically synthesized *in vitro*.
18. The process of preparing the reagent according to Claim 17 wherein the specific binding peptide is synthesized by solid phase peptide synthesis.
19. The reagent according to Claim 2 wherein the radiolabel binding moiety is covalently linked to the specific binding peptide during *in vitro* chemical synthesis.
20. The reagent according to Claim 19 wherein the radiolabel binding moiety is covalently linked to the specific binding peptide during solid phase peptide synthesis.
21. A method for labeling a peptide according to Claim 2 comprising reacting the peptide with Tc-99m in the presence of a reducing agent.
22. The method of Claim 21, wherein the reducing agent is selected from the group of a dithionite ion, a stannous ion, or a ferrous ion.
23. The reagent of Claim 1, wherein the specific binding compound comprises a cyclic peptide domain having the formula:



wherein

A is a lipophilic D- α -amino acid, or an *N*-alkyl-L- α -amino acid or L-proline;

X is an L- α -amino acid having a sidechain capable of being positively

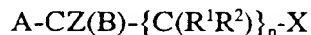
charged; and

R is each independently H, lower alkyl or lower alkoxyalkyl.

24. The reagent of Claim 23, wherein A is D-tyrosine or D-phenylalanine and X is L-(S-(3-aminopropyl)cysteine) or L-4-amidinophenylalanine.

25. The reagent of Claim 1 wherein the radiolabel complexing moiety comprises a single thiol-containing moiety of formula:

II.



wherein A is H, HOOC, H₂NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R⁴;

B is H, SH, -NHR³, -N(R³)-(amino acid or peptide), or R⁴;

X is H, SH, -NHR³, -N(R³)-(amino acid or peptide) or R⁴;

Z is H or R⁴;

R¹, R², R³ and R⁴ are independently H or lower straight or branched chain or cyclic alkyl;

n is 0, 1 or 2;

(peptide) is a peptide of 2 to about 10 amino acids;

and

where B is -NHR³ or -N(R³)-(amino acid or peptide), X is SH, and n is 1 or 2;

where X is -NHR³ or -N(R³)-(amino acid or peptide), B is SH, and n is 1 or 2;

where B is H or R⁴, A is HOOC, H₂NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH, and n is 0 or 1;

where A is H or R⁴, then where B is SH, X is -NHR³ or -N(R³)-(amino acid or peptide) and where X is SH, B is -NHR³ or -N(R³)-(amino acid or peptide);

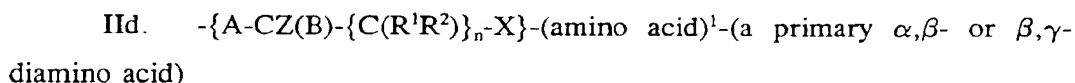
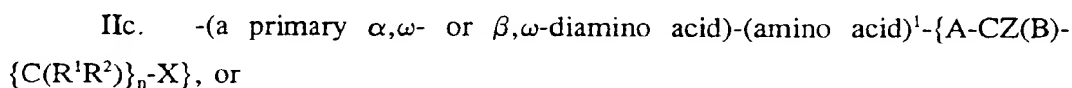
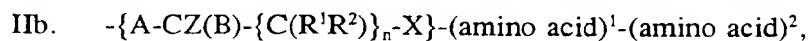
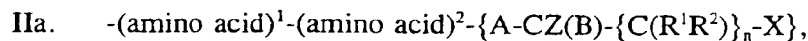
where X is H or R⁴, A is HOOC, H₂NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH;

where Z is methyl, X is methyl, A is HOOC, H₂NOC, (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, B is SH and n is 0;

where B is SH and X is SH, n is not 0;

and wherein the thiol moiety is in the reduced form and (amino acid) is any primary α - or β -amino acid not containing a thiol group.

26. The reagent of Claim 25 wherein the radiolabel complexing moiety is selected from the group consisting of the radiolabeled moieties having formula:



wherein $(\text{amino acid})^1$ and $(\text{amino acid})^2$ are each independently any naturally-occurring, modified, substituted or altered α - or β -amino acid not containing a thio, group;

A is H, HOOC, H_2NOC , (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC or R^4 ;

B is H, SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ;

X is SH or $-NHR^3$, $-N(R^3)$ -(amino acid or peptide) or R^4 ;

Z is H or R^4 ;

R^1 , R^2 , R^3 and R^4 are independently H or straight or branched chain or cyclic lower alkyl;

(peptide) is a peptide of 2 to about 10 amino acids;

n is an integer that is either 0, 1 or 2; and

where B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), X is SH and n is 1 or 2;

where X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide), B is SH and n is 1 or 2;

where B is H or R^4 , A is HOOC, H_2NOC , (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC, X is SH and n is 0 or 1;

where A is H or R^4 , then where B is SH, X is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide) and where X is SH, B is $-NHR^3$ or $-N(R^3)$ -(amino acid or peptide);

where X is H or R^4 , A is HOOC, H_2NOC , (amino acid or peptide)-NHOC, (amino acid or peptide)-OOC and B is SH;

where Z is methyl, X is methyl, A is HOOC, H_2NOC , (amino acid or peptide)-NHOC, (peptide)-OOC and B is SH and n is 0;

where B is SH and X is SH, n is not 0;

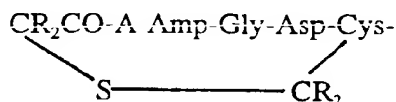
and wherein the thiol moiety is in the reduced form.

27. A composition of matter having the formula:



28. A composition of matter comprising a cyclic peptide that is less than 10,000 daltons in molecular weight and that binds to platelet glycoprotein IIb/IIIa receptor and is capable of inhibiting human platelet aggregation in platelet-rich plasma by 50% (IC_{50}) when present at a concentration of not greater than about $1\mu\text{M}$, wherein the cyclic peptide comprises the sequence -Amp-Gly-Asp-.

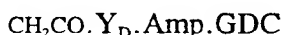
29. A composition of matter according to Claim 28, wherein the cyclic peptide comprises the formula:



wherein A is a lipophilic D- α -amino acid, or an N-alkyl-L- α -amino acid or L-proline; and

R is each independently H, lower alkyl or lower alkoxyalkyl.

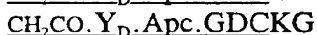
30. A composition of matter according to Claim 28, selected from the group consisting of cyclic peptides having the formula:



and



31. A composition of matter selected from the group consisting of cyclic peptides having the formula:



and O-(4-piperidiny)butyl tyrosine.

32. A composition of matter having the formula:

CH₃CO-Y_DAmpGDCKGCG.amide.

AMENDED CLAIMS

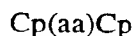
[received by the International Bureau on 02 November 1995 (02.11.95);
original claim 1 amended; remaining claims unchanged (1 page)]

1. A reagent for preparing a thrombus imaging agent for imaging thrombi within a mammalian body, comprising, in combination, a radiolabel complexing moiety that is capable of binding technetium-99m, indium-111 or gallium-68, and that is covalently linked to a specific binding compound that is less than 10,000 daltons in molecular weight and that binds to a platelet glycoprotein IIb/IIIa receptor, wherein the reagent is capable of inhibiting human platelet aggregation in platelet-rich plasma by 50% (IC_{50}) when present at a concentration of not greater than about $1\mu M$.

2. A reagent according to Claim 1 wherein the specific binding compound is a specific binding peptide comprising 4 to 100 amino acids.

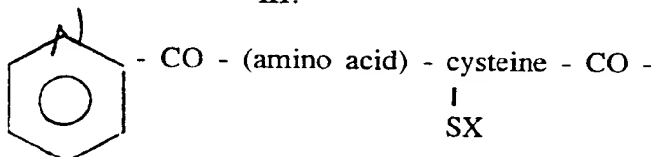
3. The reagent of Claim 1 wherein the radiolabel complexing moiety has a formula selected from the group consisting of:

I.

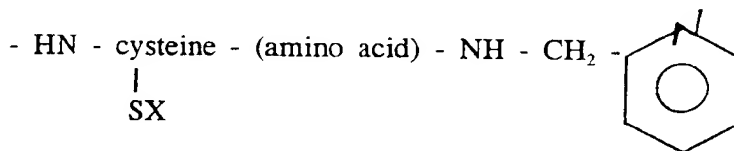


wherein Cp is a protected cysteine and (aa) is any primary α - or β -amino acid not containing a thiol group; and

III.



IV.



wherein

X = H or a protecting group;

(amino acid) = any primary α - or β -amino acid not containing a thiol group;

Figure 1
Sheet 1 of 2

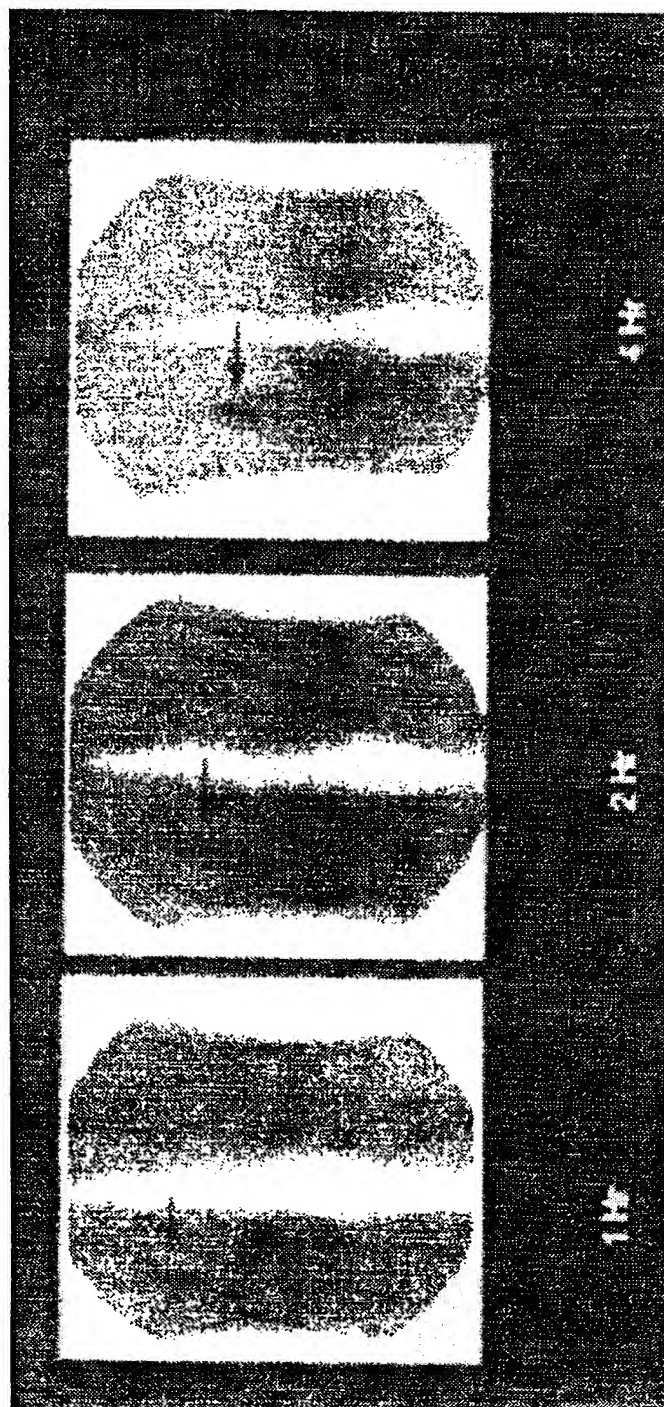
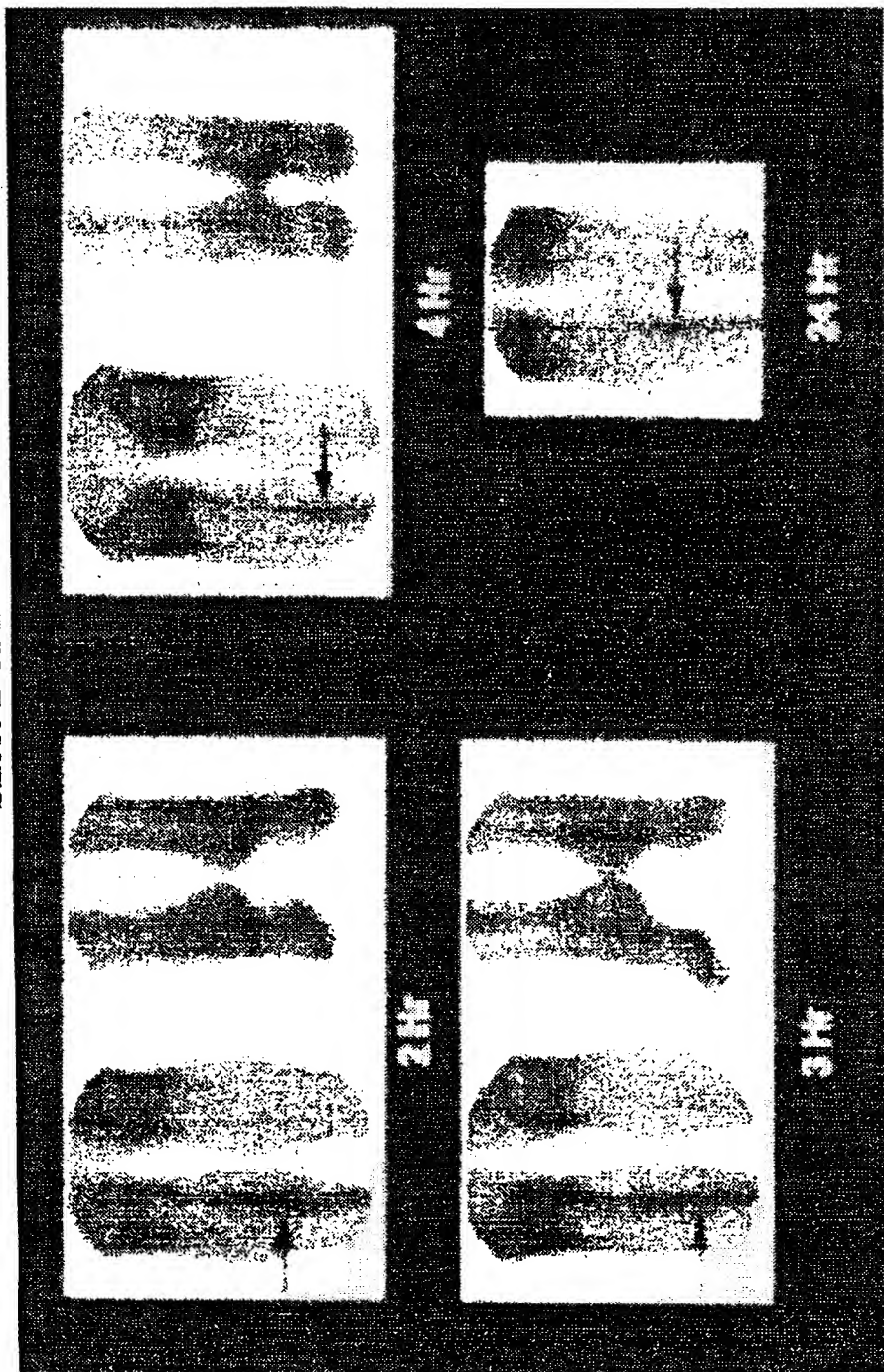


Figure 2

Sheet 2 of 2



INTERNATIONAL SEARCH REPORT

Inter. Appl. Application No
PCT/US 95/06909

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 A61K51/08		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,94 23758 (DIATECH) 27 October 1994 cited in the application see abstract see page 12, line 1 - page 13, line 29; claims; examples; tables I,II ---	1-32
X	WO,A,93 23085 (DIATECH) 25 November 1993 cited in the application see the whole document ---	1-27,31
X	WO,A,93 25244 (DIATECH) 23 December 1993 cited in the application see abstract see page 7, line 1 - page 14, line 6; claims; examples 4,5; table I --- -/--	1-27,31
<div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. </div> <div> <input checked="" type="checkbox"/> Patent family members are listed in annex. </div> </div>		
* Special categories of cited documents : <div style="display: flex;"> <div style="flex: 1;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search 24 August 1995		Date of mailing of the international search report 05.09.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016		Authorized officer Hoff, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06909

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,93 21962 (DIATECH) 11 November 1993 cited in the application see the whole document, in particular page 31 line 7 and example 5 ---	1-27,31
X	THE JOURNAL OF NUCLEAR MEDICINE, vol. 34, no. 5, May 1993 pages 17P-18P, LC KNIGHT ET AL. 'EVALUATION OF TC-99M LABELED CYCLIC PEPTIDES FOR THROMBUS IMAGING' see the whole document ---	1-14, 16-20, 23,24, 27,31
X	WO,A,94 07918 (DIATECH) 14 April 1994 cited in the application see the whole document ---	1-6, 9-11, 23-27,31
A	WO,A,93 10747 (DIATECH) 10 June 1993 cited in the application see the whole document ---	1-32
A	WO,A,92 13572 (DIATECH) 20 August 1992 cited in the application see the whole document ---	1-32
A	WO,A,90 15818 (ANTISOMA) 27 December 1990 cited in the application see the whole document -----	1-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/ 06909

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
please see enclosed sheet ../..

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

INCOMPLETE SEARCH

2. Obscurities, Incnsistencies,...

A compound cannot be sufficiently characterised by its pharmacological profile on its mode of action as it is done by expressions like "specific binding compound...that binds to a platelet receptor..." or "...capable of inhibiting human platelet aggregation".

In view of the large number of compounds which are defined by the general formulas of claims 3,25-26, the search was limited to the inventive part of the molecules and to the compounds mentioned in the examples and in the claims 9,27,30-32.

claims searched completely: 9,27,30-32

claims " incompletely: 1-8,10-26,28,29

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 95/06909

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9423758	27-10-94	AU-B- 7241394	08-11-94
WO-A-9323085	25-11-93	AU-B- 4384593	13-12-93
		CA-A- 2136330	25-11-93
		EP-A- 0641222	08-03-95
WO-A-9325244	23-12-93	AU-B- 4528793	04-01-94
		CA-A- 2137009	23-12-93
		EP-A- 0644778	29-03-95
		JP-T- 7506592	20-07-95
WO-A-9321962	11-11-93	AU-B- 4107693	29-11-93
		CA-A- 2111863	11-11-93
		EP-A- 0637968	15-02-95
		JP-T- 7506111	06-07-95
WO-A-9407918	14-04-94	AU-B- 5402694	26-04-94
		CA-A- 2145847	14-04-94
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WO-A-9213572	20-08-92	AU-B- 658617	27-04-95
		AU-A- 1411792	07-09-92
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		JP-T- 4505330	17-09-92
		US-A- 5395609	07-03-95